Behavior and Adherence to Black Raspberry Functional Confections Used in Oral Health Promotion

Thesis

Presented in Partial Fulfillment of the Requirements for Undergraduate Honors Research Distinction in the Ohio State University College of Food, Agricultural, and Environmental Sciences

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Abstract

Historically, dental health research has been largely isolated from overall health research. However, in recent years, the role of good dental health as part of an overall healthy lifestyle has become more prominent. Black raspberries (BRB) contain key bioactive and health- benefitting compounds (ellagitanins and anthocyanins) associated with oral health promotion. This study analyzed data obtained from a human clinical trial conducted at the Ohio State University, in which 60 healthy adults (30 men and 30 women) consumed one of three amorphous black raspberry confections every day for two weeks at 2 different BRB doses (4g and 8g). We hypothesized that subjects participating in this study with poor diet and poor dental hygiene practices would also have poor adherence to the confection intervention. Additionally, we hypothesized that the confection matrix with the longest exposure in the oral cavity would yield the greatest concentration of total urolithins (metabolites of ellagitanins) excreted in the urine. The objectives of this research were to correlate lifestyle and modifiable behaviors with adherence to the dietary intervention and to determine difference in BRB exposure among the three BRB confections. We examined health and lifestyle questionnaires, adherence data, and quantifying total urolithin levels by HPLC MS in 24 hr. urine samples obtained from the 60 participants in the oral health promotion study. No correlation could be made between modifiable behaviors and compliance to the diet intervention. From the three confection matrices tested in this trial, the high dose (8g BRB/ piece) hard candy yielded the highest amount of urolithins in participant urine (4,695±9161 nmol/24 hr.). This work is important in identifying the optimal delivery vehicle of BRB for future clinical trials.

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I would like to thank Dr. Yael Vodovotz for welcoming me into her research group and for allowing me to work on this project from an early age. Being a part of her group has given me the opportunity to see what research is all about and to learn from the other members of her group, as well as inspired me to continue in her lab to complete my Master's of Food Science degree. It has been an invaluable experience. I would also like to thank Dr. Jennifer Ahn-Jarvis for her hands on guidance, instruction, and mentorship throughout my time working on this project. This project would not have been possible without her continued direction and support.

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Introduction

An emerging area of research within dental science is the promotion of dental health and its correlation to a person's health overall.[1] When looking at dental health, traditional oral hygiene habits should be closely examined, since poor dental hygiene behaviors can be indicators of future dental disease risk.[1] It has been shown that dental diseases, such as dental carries, may themselves not be fatal, but can cause indirect health consequences such as an impairment in chewing function subsequently preventing consumption of a balanced diet, in turn decreasing quality of life.[1]

The World Health Organization/Food and Agriculture Organization dietary recommends to increase the consumption of fruits and vegetables and wholegrain foods to prevent a number of chronic conditions, including obesity, CVD, cancer and diabetes[1]. In Western countries, modifiable behaviors such as a diet with fruit and vegetable intake less than the RDA may also be responsible for up to 20-25% of oral and pharyngeal cancers.[2] Black raspberries (BRB) are food ingredients of great interest in nutrition research because of their complexity of bioactive compounds and their demonstrated efficacy in clinical trials with oral and pharyngeal cancer (OPC).[3] BRB contain a high concentration of polyphenols and other bioactives known to inhibit oral carcinogenesis.[3] Specific for this study is the fact that BRB contain a high concentration of a specific class of polyphenols: ellagitannins and ellagic acid. Other ellagitannin-containing foods (strawberries, walnuts, pomegranate, raspberries, oak-aged wine, etc.) have been studied for their cancer chemopreventive, cardioprotective, and antioxidant effects[3]. Ellagitannins (ETs) are not absorbed by the body as such but are metabolized by the intestinal flora to yield urolithins A and B which can be quantified through urine collection[4, 5].

Since consuming fresh black raspberries is not feasible year round, a highly acceptable food vehicle containing concentrated black raspberry powder was needed. Three different amorphous black raspberry confections were formulated as the delivery method for the BRB bioactive compounds in clinical trials at The Ohio State University to discriminate the impact of amorphous food structure on the release rate and duration of BRB exposure in the oral cavity.[5] Hard candies had the longest release followed by the starch gummy with intermediate release rate, and finally followed by the rubbery pectin gummy having the slowest release rate [6,7]. Fisher and colleagues (2014) reported that functional starch confections release rates found from laboratory dissolution experiments were different from oral dissolution among human participants [8]. Therefore, we anticipated that the three confections would show differences in BRB release rates as well as different amount of residence times in the human oral cavity compared to *ex vivo* dissolution studies.

Problem Identification and Justification

Both oral hygiene practices as well as modifiable behaviors have been identified as having a significant correlation with tooth loss and decreasing oral health through recent academic studies. Examples of these hygiene practices and behaviors include frequency of between-meal snacks, alcohol consumption, smoking, frequency of tooth brushing, having some hobbies, having a family dentist and consulting a dentist when dental symptoms such as bleeding gums or toothache occur[9]. The intent of this study was to assess oral health indicators utilizing a Health and Lifestyle questionnaire in healthy adults that would subsequently consume BRB confections equivalent to a serving of 1/2 cup of BRB a day. Adherence was measured to identify whether the participants simply

were or were not consuming the confections or if they were not being metabolized within the body through the measure of urolithins excreted in the urine. Aside from health status, confection matrix may also lead to different absorption and metabolism of the ellagitanins found in the BRB. Therefore in this investigation the working hypothesis was that those with poor diet and poor dental hygiene practices would also have poor adherence to the intervention confections. Also, it was hypothesized that the confections with the longest exposure in the oral cavity would yield the greatest concentration of total urolithins excreted in the urine. The following objectives were used to test the hypothesis:

Objectives

These objectives will be met from the analysis of data obtained from a month long oral health promotion study of 60 adults at the Ohio State University using three different amorphous BRB confections. Daily diaries, health and lifestyle questionnaires, and participant urine analyzed by HPLC will be the clinical study data used to execute these objectives.

1. To correlate lifestyle and modifiable behaviors with adherence to the dietary intervention. Daily diaries were used to measure self-reported compliance to dietary interventions as well as a health and lifestyle questionnaire were used to collect lifestyle and behavioral information. Data was correlated to determine if a relationship exists between healthy lifestyles and those having high compliance throughout the clinical trial.
2. To determine difference in BRB exposure among the three BRB confections. Total urolithins excreted in the urine were used to determine the impact of amorphous structure on oral exposure of bioactive compounds.

Materials and Methods

Clinical Study Design

This study (Figure 1) was conducted at the Ohio State Clinical Research Center (CRC) Center for Clinical and Translational Sciences, and required three visits to the CRC (IRB protocol #2013C0056)**.** 60 total subjects, 30 men and 30 women, were selected for participation in this 4 week study which included a 2 week berry washout, followed by two weeks of BRB intervention. Once a subject was selected for participation in the oral health study, they were scheduled for an initial appointment, designated Day -14, with a study coordinator where they were instructed on how to complete a low berry diet to adhere to throughout the study, as well as how to complete a health and lifestyle questionnaire and daily compliance forms. After the two-week washout, the participants returned to the CRC for a random assignment to one of the six possible BRB treatment plans. Baseline blood, buccal, and urine samples were taken by RNs on Day 0, and the supply of the assigned BRB confections was distributed for the two-week intervention portion of the study. Each participant received the assigned berry confection containing 1.3 grams of BRB powder. Participants consumed either 3 or 6 confections per day (4g or 8g BRB), which is equivalent to ~0.5 or 1 serving of fresh black raspberries per day. Each participant was given detailed instructions on how to consume the confection for the two-week intervention (the pieces should be consumed approximately 4-6 hours apart). At the final visit to the CRC on Day 14, the CRC staff RNs assisted in collecting blood, buccal, and urine from the participant.

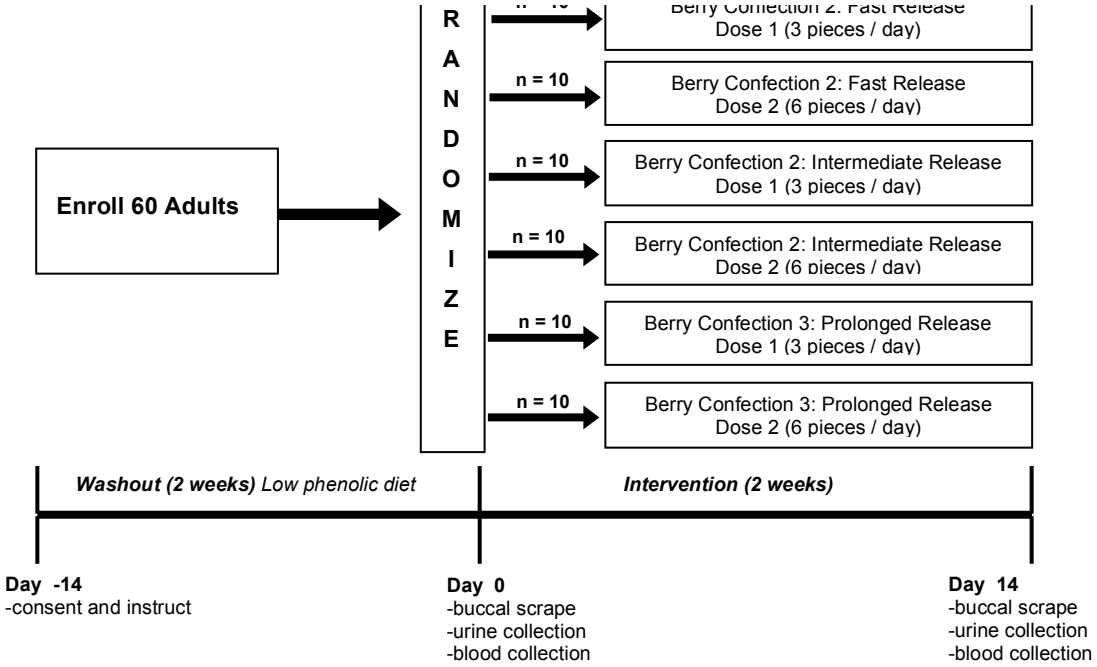

Figure 1. Schematic of clinical study

Cohort description

A sample size of 60, 30 women and 30 men, was chosen to participate in the clinical study based on the following criteria:

***Inclusion Criteria*:**

- Be healthy, free-living adults 18 to 65 years of age
- Have a body mass index (BMI) between 18 and 35kg/m^2
- Be a non-smoker (defined as adults who have never smoked or who have not had a cigarette in the past ten years)
- Agree to consume a standardized vitamin/mineral supplement and avoid other nutrition and alternative supplements for the duration of the study
- Agree to follow a berry-restricted diet and to document any accidental consumption of restricted foods (see Appendix) each day of the study
- Agree to abstain from mouthwashes

***Exclusion Criteria*:**

- Have an active metabolic or digestive illness including malabsorptive disorders, renal insufficiency, hepatic insufficiency, cachexia, or short bowel syndrome
- Have an active or a recent history of any condition that causes altered immunity such as chronic inflammatory disease, autoimmune disorders, cancer, anemia, and blood dyscrasias
- Have a known allergy or food intolerance to ingredients in study products (black raspberries), other berries, wheat, or soy
- Are strict vegans (no consumption of animal, fish or egg products)
- Are planning to conceive, or are currently pregnant or lactating
- Are heavy alcohol consumers (defined as an average consumption of greater than 2 drinks/ day)
- Have a history of oral cancer or currently undergoing treatment of oral cancer
- In the last month have had any active oral lesions or maladies or currently have any oral disease or obvious open sores in the oral cavity or surrounding the oral opening.
- Have strong gag reflex or problems swallowing that prohibit buccal brushing of the oral cavity
- Have been on an antibiotic regime lasting for one week in the last 6 months

Preparation of Materials

Description of confection preparation

The black raspberry confections were produced at The Ohio State University Food Industries Center Gould Food Pilot Plant using the guidelines and procedures of safe food processing. Three different amorphous forms were evaluated: starch gummy, pectin gummy, and a hard candy. The confection base included freeze-dried whole black raspberries (Stokes Farm, Wilmington, OH). The hard candy will be produced using corn syrup (Gordon Foods, Grand Rapids, MI), sugar (sucrose; US food service, Cincinnati, OH) while pectin and starch confections contained sugar, corn syrup, freeze-dried BRB powder, and gelling agents- Grindsted pectin (70% esterification CF 130B, Danisco USA Inc., New Century KS) and Confectioners G food starch (Tate & Lyle, Decatur IL). The pectin-based and starch-based confections were prepared by combining water, sugar, corn syrup and gelling agent, and stirring the mixture or a stirrer/hotplate (PC-620D, Corning, Tewksbury, Mass., U.S.A.) to a final temperature of 95°C and °Brix of 67± 2, was determined by a hand-held refractometer (range 58 to 90 °Brix, Fisher Scientific Japan Ltd., Tokyo, Japan). The mixture was cooled at room temperature to about 70°C so the freeze-dried BRB powder could be added. The mixture was deposited through a pastry bag onto a Silpat (Demarle, Inc. Cranbury, NJ). Confections were equilibrated at room temperature in the dark for 24 hours, and then packaged into 1 or 2 oz. plastic portion cups with lids (Gordon Food Service). The hard candy was prepared by heating a mixture of corn syrup, sugar, and water to 150°C and BRB powder was directly added to the mixture and blended. Confections were then deposited onto the Silpat and set and packaged the same way the gel gummies. All confections were prepared under a relative humidity between 22% to 42% and ambient temperatures (19 °C to 22 °C).

Study Instruments

H & L questionnaire

One of the instruments utilized in this study was data collected from a health and lifestyle questionnaire. The data collected helped to gain a better understanding of the regular behaviors of study participants. This health and lifestyle questionnaire is composed of questions about biographical information such as age, gender, race, and occupation. The questionnaire also helps to obtain information about the participant's diet and exercise habits, medical history, dental/oral health history, smoking history, and alcohol consumption.

Daily Journal

Another resource of data that was investigated was a daily journal. In this daily compliance journal participants completed throughout the two-week BRB intervention. The journal detailed the number of confections consumed, the times they were consumed, as well as the time it took for the confection to completely dissolve in the mouth. Finally, in the daily journal there was also a place to note if any restricted foods were eaten throughout the two-week intervention that would not comply with study instructions.

Total Urolithins from Urine

Total urolithins from 24 hour urine collection was used for this proposed study to compare presence of urolithins with a participant's self-reported compliance to the diet restriction and study intervention as well differences in total urolithins among the three confectionary matrices will be examined. For the 24-hour urine collection, patients were instructed to collect a total of two 24-hour urine samples. These collections were completed on Day -1 and Day 13 and brought to CRC clinic visits the day following each

collection. Participants were instructed by the RNs at the CRC on the correct method to collect a 24-hour urine. The total volumes were recorded, then aliquoted into six, 5 ml tubes and stored at -80° C.

Extraction of water-soluble compounds from urine was assisted by addition of acidified methanol. Once polyphenolic compounds were isolated, extracts were further separated into more polar compounds by reversed phase HPLC on a C_{18} column employing an acidified water/acetonitrile gradient. The Nutrient and Phytochemical Analytical Shared Resource conducted quantification and characterization of total urolithins from urine using HPLC with tandem mass spectroscopy.

Statistical Analysis

Fisher exact test was used to determine significant modifiable behaviors, which attribute to healthy versus unhealthy oral hygiene and a Pearson correlation was used to correlate these modifiable behaviors with adherence to study intervention. To determine difference in BRB exposure, a one-way ANOVA analysis was used to compare the total urolithin excreted among the three confections.

Results and Discussion

67 total people were recruited for this clinical study, but due to seven people dropping out, only 60 competed the two-week washout period along with the two-week confection intervention. The median age of the cohort was 33±11 years old, and most of the cohort (>50%) maintained healthy behaviors in their daily lives such as: not smoking, no dental surgery, twice a day tooth brushing, and daily flossing (Table 1.) Regular alcohol consumption was observed in ~70% of the participants compared to 56.9% in the US population[11]. Unfortunately, due to the descriptive nature of this data, no statistical

correlation between the behaviors could be made with compliance to the confections as to satisfy the initial objective of this work.

Table 1. Demographic Data of Clinical Study Cohort

Demographic Measure	Result
Age (mean ± SD)	33±11 years old
BMI (mean ± SD)	26.6 ± 9.2 kg/m^2
Regular alcohol consumers (3.25 servings/ week)	69% (46/67)
Past Smokers (0.5 pack/ 5.5 years)	11% (7/67)
Never smokers	89% (60/67)
History of Dental Surgery	13% (9/67)
Twice Daily Teeth Brushing	66% (44/67)
Daily Flossing	69% (46/67)

In table 2 below, the results for the self-reported compliance data are shown, and this data was obtained from the participants' daily journals. Overall, very good compliance can be seen among all three confections (>90%), suggesting that any difference in urolithin levels in the participants' 24-hour urine is due to variances in confection matrix (i.e. hard, pectin, or starch) rather than differences in the frequency of eating the candies. The high levels of compliance among all confections show promise for the possibility of using these same confections in future clinical trials.

Table 2. Compliance Data of Clinical Study Cohort

Confection	Compliance (mean ±SD)
Hard candy	93.2±15.0%
Pectin candy	93.9±12.8%
Starch candy	96.4±12.3%

No statistical difference in level of urolithins in the 24-hour urine among the three confections was observed in subjects given the low dose confections (4g BRB/ day or 3 pieces of candy/ day) (Figure 2).

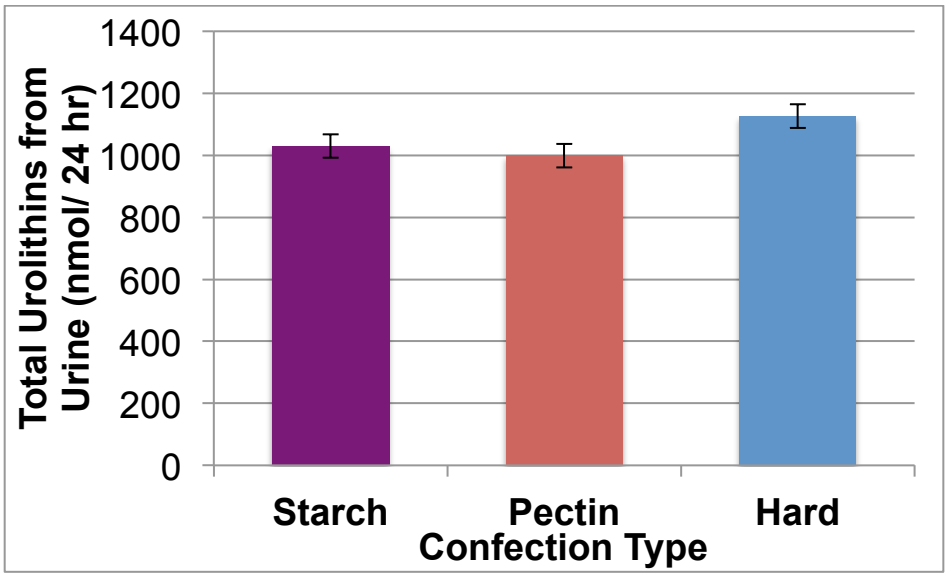

Figure 2. Total urinary urolithin from low dose (4g BRB/day or 3 pieces/day) confections.

Figure 3 below displays the 24-hour urolithin data for the high dose (8 g BRB/ day or 6 confections/ day) BRB confections. However, unlike in the case of the low dose confections, a statistical difference in urolithin concentration in the 24-hour urine was observed among the pectin, starch, and hard candies. Subjects consuming high dose BRB

hard candy for two weeks had the highest concentration of urolithins in their 24-hour urine sample. The next highest concentration was seen in the portion of the cohort who took the high dose starch confections, followed lastly by the high dose pectin BRB candies.

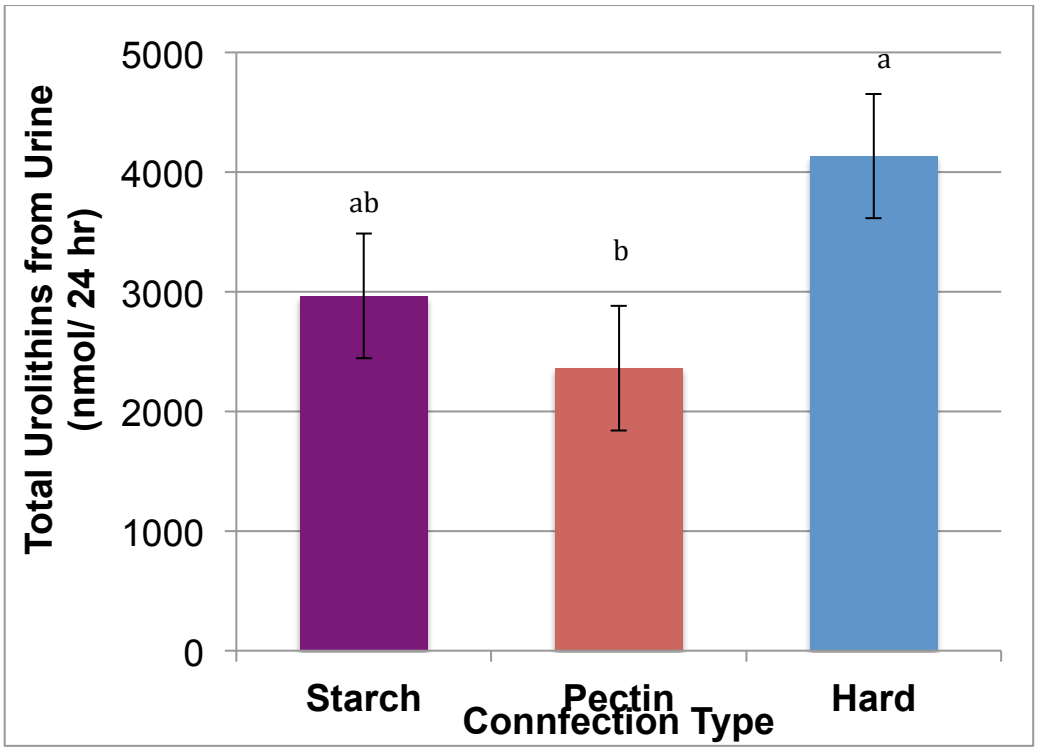

Figure 3. Total urolithin from high dose (8g/day or 6 pieces/day) confections from 24-hour urine. One way ANOVA with Tukey's posthoc test was used to discriminate significant differences (p=0.023)

Conclusion

In conclusion, functional confections are an excellent strategy for delivery of BRB compounds having excellent compliance in future long-term clinical trials. Differences in total urolithins in high dose BRB confection suggest that confection matrix may impact BRB bioactive metabolism, with the high dose (8gBRB/day) hard candies lead to the highest concentration of urinary urolithins.

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